

Investigator

William McClements

Date

9 Oct 97

Subject

HPV L2 primers

Filed in Book Number/Title

WLM XVIII / w/m 97.14

9 Oct 97

Design PCR primers to allow generation of L2 vectors for testing directly in VLP or for fusing with E2 proteins for testing VLPs

1. will introduce unique Not I, SacI, and XhoI sites in L2 (in pGal110 HPV16 L1+L2)

a. See attached material for evaluation of flanking

b. Pvt use is may be dangerous, pGal110 HPV16 L1+L2 appears partially "niche & key" but I see JCL

2. primers ordered 9.10.97

MIDLAND FAXORDERFORM

Billing Information: Customer #40

Shipping Information: Deliver To end user

End-User Name:

William McClements
WP26-251

Phone 215 652 7861

FAX (7320)

Merck Research Laboratories 8 Oct. 1997
West Point, Pa. 19486

Ordering Person:

William McClements

Phone 215 652 7861

P.O. Number to be generated

Purity DRY EP GF AE PAGE Other

Ship Day M T W T F S any

USPS

FDX

USM

Quantity 1 2 3 5 10 other ODU all

13856-233a

47mer

5' CCT ACC GGC GGC CGC GAG CTC GAG GGT TAT ATT CCT GCA AAT ACA AC

13856-233c

27mer

5' GGG ACC GCG GCC GCC TGT ACC CGA CCC

13856-233d

35mer

5' CCC TCC AGA CTC CTA GGC AGC CAA AGA GAC ATC TG

13856-233f

41mer

5' GAC TAC GGC GGC CGC GAG CTC ACT GGG TAT ATT CCA TTG GG

13856-233g

33mer

5' CAC GAC CTC GAG AGA TGT AGA GGG TAC AGA TGG

13856-233h

41mer

5' CGC CGC GGC GGC CGC GAG CTC CCA GCA TTA ACC TCT AGG CG

13856-233i

28mer

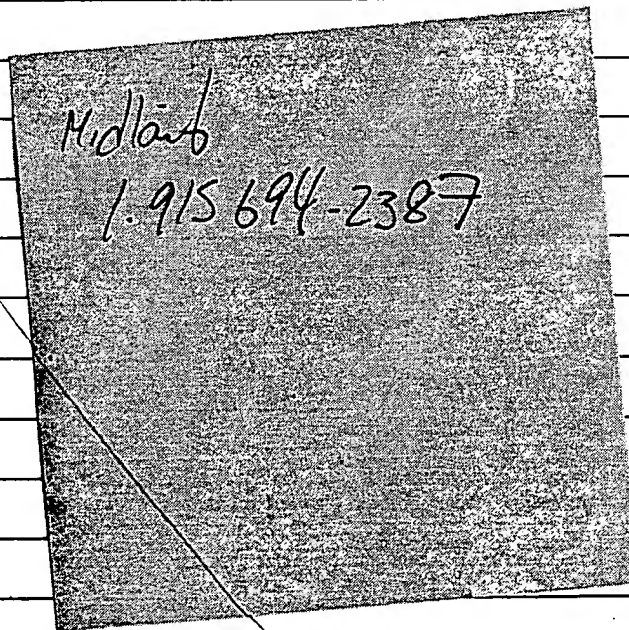
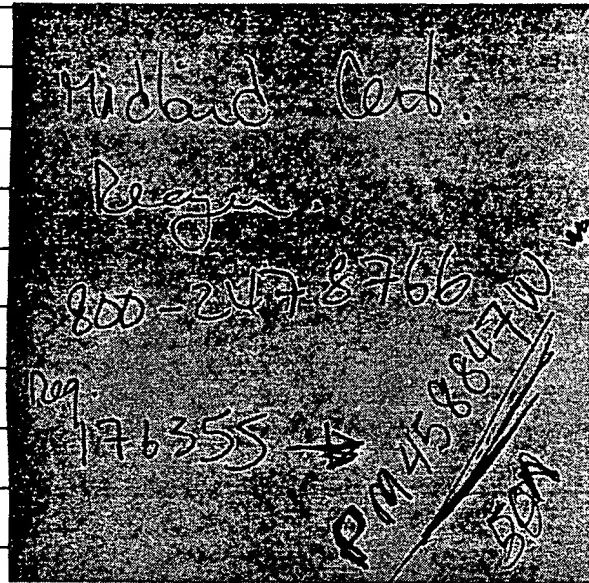
5' CT TCC CCC CGG GCA CAA AAC AAA ATG C T

William McClements 9 Oct 97

Countersigned by

Date

DO NOT BEGIN NEW EXPERIMENTS ON THIS PAGE.



Investigator

Jessica Ling

Date

15 Oct 97

Subject

PCR of N-Terminus + C-Terminus of HPV16 L2

Filed in Book Number/Title

Ling HPV-1 / 97-7 and 97-8 JCL 16 Oct 97

Objective: to amplify via PCR the N-Terminus and C-Terminus of HPV16-L2 for creation of constructs. Refer to attachments for primer location & schematic.

15 Oct 97 Dilute primers from midland. See accompanying pages for Certificates of Analysis. Resuspend w/ dH₂O. Dilute to 100 μ M = 100×10^{-6} moles = 100×10^{-6} moles

$$= 100 \times 10^{-12} \text{ moles} / \mu\text{L}$$

RCM66 #M1-7113

13856-233A 56.3 nmoles = 563 μ L

13856-233C 73.2 nmoles = 732 μ L

13836-233D 89.3 nmoles = 893 μ L

13856-233F 94.1 nmoles = 941 μ L

13836-233G 57.9 nmoles = 579 μ L

13856-233H 53.9 nmoles = 539 μ L

13856-233I 54.0 nmoles = 540 μ L

These are 100 μ M stocks

Working stocks are 5 μ M (1:20) w/ dH₂O

Setup per to amplify I+C and A+D fragments.

Component [Final]	1x Volume	3.5x Volume
D125-DNA [0.1 μ g/ λ]/50	1 μ L	3.5 μ L
10x Buffer [1x]	5 μ L	17.5 μ L
25m MgCl ₂ [1.5 mM MgCl ₂]	3 μ L	10.5 μ L
10mM dNTPs [0.2 μ M ea]	4 μ L	14 μ L
5 μ M primer (1) [0.2 μ M]	2 μ L	—
5 μ M primer (2) [0.2 μ M]	2 μ L	—
tag gold [2.5 U/ μ l]	0.5 μ L	1.75 μ L
dH ₂ O	32.5 μ L	113.75 μ L

/align
/46 λ / tube

Countersigned by

Pauline Mudgett

Date

22 Oct 97

DO NOT BEGIN NEW EXPERIMENTS ON THIS PAGE.

Jessie L.
16 Oct 97

Investigator

Jessica King

Date

03 Dec 97

Subject

Seq Rxns for P3 and Z7

Filed in Book Number/Title

249 HPR-1 / JCL 97 113

Setup for Seq rxns for P3 and Z7

P3 [1.2 ug/l] 1 : 4.8 = 2ul DNA + 7.2ul dH₂O

Z7 [0.3 ug/l] 1 : 1.2 = 8ul + 1.6ul

Setup

M13

16A11

DNA

3ul

3ul

primer

1.1ul

3.2ul

tag ES

4ul

4ul

dH₂O

11.9ul

9.8ul

Also sequence

M13/dH₂O16A11/dH₂O

Z3 [0.18 ug/l]

4.2ul / 10.7ul

8.6ul / 4.2ul

Z4 [0.166 ug/l]

4.4ul / 10.5ul

8.4 / 4.4ul

P6 [0.187 ug/l]

3.9ul / 11ul

8.9 / 3.9ul

P7 [0.194 ug/l]

3.9ul / 11ul

8.9 / 3.9ul

Jessica King
03 Dec 97

Countersigned by

Corinne D. Bitt

Date

22-Jan-98

DO NOT BEGIN NEW EXPERIMENTS ON THIS PAGE.

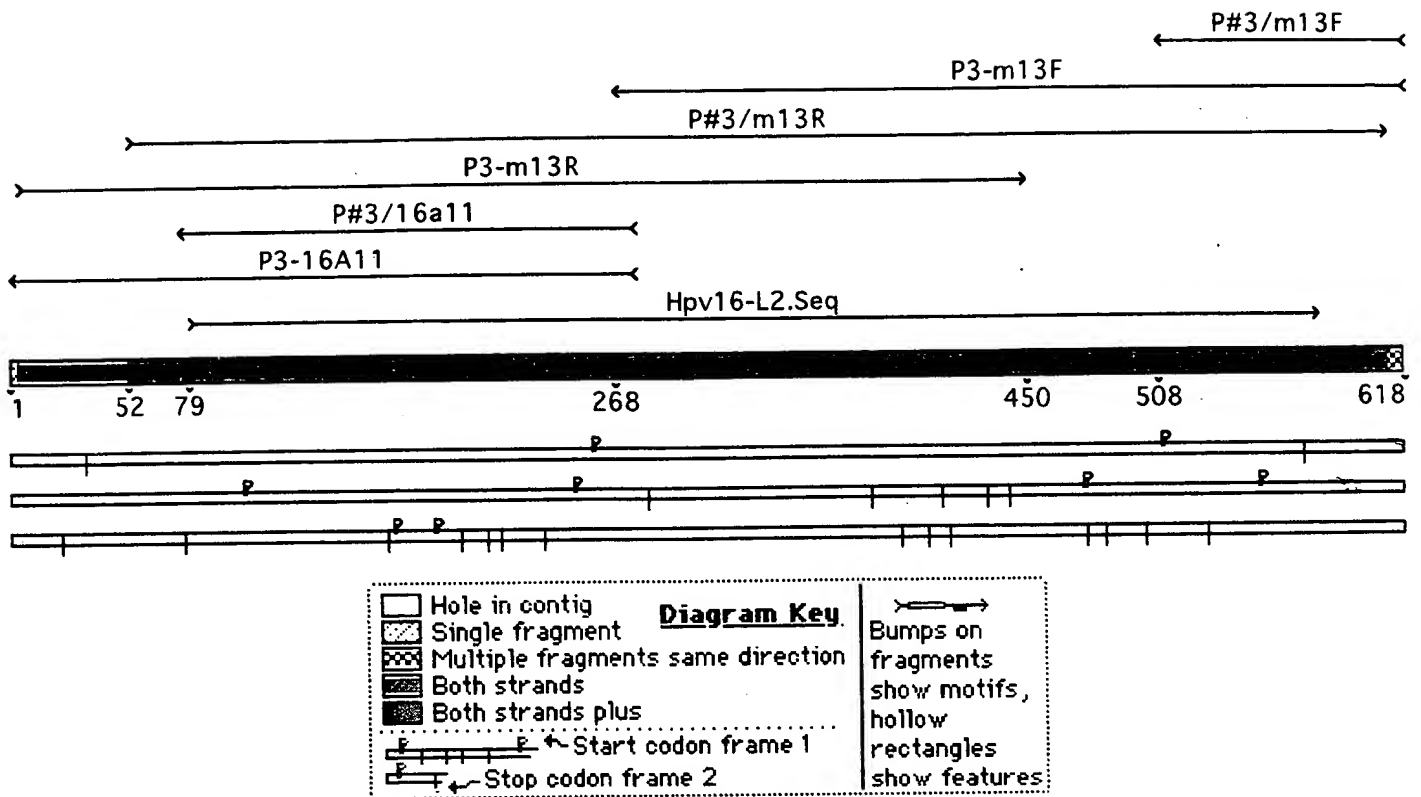
Results:

Still the primers as in the case the last time did not extend very well. But P3 was completely sequenced in two directions. So will continue with this clone. Also 27 was completely sequenced. Will also continue w/ this clone.

Both will be transformed into the yeast vector

Jessica
03 Dec 97

P#3 Clone
Sequencher™ "Min. vector"



P#3 Clone
Sequencher™ "Min. vector"

↓ P#3/m13R

↓ P3-m13R

↓ P3-16A11

#1

ACGCCA AGCGGCGCAAT TAACCCCTCAC TAAAGGGAAC AAAAGCTGGA GCTCCACCGC GGTGGCGGGCC
GATTACGCCA AGCGGCGCAAT TAACCCCTCAC TAAAGGGAAC AAAAGCTGGA GCTCCACCGC GGTGGCGGGCC

GATTACGCCA AGCGGCGCAAT TAACCCCTCAC TAAAGGGAAC AAAAGCTGGA GCTCCACCGC GGTGGCGGGCC

↓ P#3/m13R

↓ P3-m13R

↓ P#3/16a11

↓ P3-16A11

↓ Hpv16-L2.Seq

#71

GCTCTAGCCC CTTCCCCCCC GGCACAAAAC AAAATGCGAC ACAACGGTTC TGCAAAACGC ACAAACCGTG
GCTCTAGCCC CTTCCCCCCC GGCACAAAAC AAAATGCGAC ACAACGGTTC TGCAAAACGC ACAAACCGTG
AGCCC CTTCCCCCCC GGCACAAAAC AAAATGCGAC ACAACGGTTC TGCAAAACGC ACAAACCGTG
GCTCTAGCCC CTTCCCCCCC GGCACAAAAC AAAATGCGAC ACAACGGTTC TGCAAAACGC ACAAACCGTG
CTTCCCCCCC GGCACAAAAC AAAATGCGAC ACAACGGTTC TGCAAAACGC ACAAACCGTG

GCTCTAGCCC CTTCCCCCCC GGCACAAAAC AAAATGCGAC ACAACGGTTC TGCAAAACGC ACAAACCGTG

↓ P#3/m13R

↓ P3-m13R

↓ P#3/16a11

↓ P3-16A11

↓ Hpv16-L2.Seq

#141

CATCGGCTAC CCAACTTTAT AAAACATGCA AACAGGCAGG TACATGTTCA CCTGACATTA TACCTAAGGT
CATCGGCTAC CCAACTTTAT AAAACATGCA AACAGGCAGG TACATGTTCA CCTGACATTA TACCTAAGGT
CATCGGCTAC CCAACTTTAT AAAACATGCA AACAGGCAGG TACATGTTCA CCTGACATTA TACCTAAGGT
CATCGGCTAC CCAACTTTAT AAAACATGCA AACAGGCAGG TACATGTTCA CCTGACATTA TACCTAAGGT
CATCGGCTAC CCAACTTTAT AAAACATGCA AACAGGCAGG TACATGTTCA CCTGACATTA TACCTAAGGT

CATCGGCTAC CCAACTTTAT AAAACATGCA AACAGGCAGG TACATGTTCA CCTGACATTA TACCTAAGGT

P#3 Clone Sequencher™ "Min. vector"

1 P3-m13F

1 P#3/m13R

1 P3-m13R

1 P#3/16a11

1 P3-16A11

1 Hpv16-L2.Seq

#211

```

TGAAGGCCAAA ACTATTGCTG ATCAAAATATT ACAAATATGGA AGTATGGGTG TATTTTGG TGGGTTAGGA
TGAAGGCCAAA ACTATTGCTG ATCAAAATATT ACAAATATGGA AGTATGGGTG TATTTTGG TGGGTTAGGA
TGAAGGCCAAA ACTATTGCTG ATCAAAATATT ACAAATATGGA AGTATGGGTG TATTTTGG TGGGTTAGGA
TGAAGGCCAAA ACTATTGCTG ATCAAAATATT ACAAATATGGA AGTATGGGTG TATTTTGG TGGGTTAGGA
TGAAGGCCAAA ACTATTGCTG ATCAAAATATT ACAAATATGGA AGTATGGGTG TATTTTGG TGGGTTAGGA
TGAAGGCCAAA ACTATTGCTG ATCAAAATATT ACAAATATGGA AGTATGGGTG TATTTTGG TGGGTTAGGA
TGAAGGCCAAA ACTATTGCTG ATCAAAATATT ACAAATATGGA AGTATGGGTG TATTTTGG TGGGTTAGGA
TGAAGGCCAAA ACTATTGCTG ATCAAAATATT ACAAATATGGA AGTATGGGTG TATTTTGG TGGGTTAGGA

```

1 P3-m13F

1 P#3/m13R

1 P3-m13R

1 Hpv16-L2.Seq

#281

```

ATTGGAACA: GGTCGGGTAC AGGCGGCCGC GAGCTCGAGG GTTATATTCC TGCAAAATACA ACAATTCCTT
ATTGGAACAG GGTCGGGTAC AGGCGGCCGC GAGCTCGAGG GTTATATTCC TGCAAAATACA ACAATTCCTT
ATTGGAACAG GGTCGGGTAC AGGCGGCCGC GAGCTCGAGG GTTATATTCC TGCAAAATACA ACAATTCCTT
ATTGGAACAG GGTCGGGTAC AGGCGGCCGC GAGCTCGAGG GTTATATTCC TGCAAAATACA ACAATTCCTT
ATTGGAACAG GGTCGGGTAC AGGCGGCCGC GAGCTCGAGG GTTATATTCC TGCAAAATACA ACAATTCCTT
ATTGGAACAG GGTCGGGTAC AGGCGGCCGC GAGCTCGAGG GTTATATTCC TGCAAAATACA ACAATTCCTT
ATTGGAACAG GGTCGGGTAC AGGCGGCCGC GAGCTCGAGG GTTATATTCC TGCAAAATACA ACAATTCCTT

```

1 P3-m13F

1 P#3/m13R

1 P3-m13R

1 Hpv16-L2.Seq





#351

```

TTGGTGGTGC ATACAATATT CCTTTAGTAT CAGGTCCTGA TATACCCATT AATATAACTG ACCAAGCTCC
TTGGTGGTGC ATACAATATT CCTTTAGTAT CAGGTCCTGA TATACCCATT AATATAACTG ACCAAGCTCC
TTGGTGGTGC ATACAATATT CCTTTAGTAT CAGGTCCTGA TATACCCATT AATATAACTG ACCAAGCTCC
TTGGTGGTGC ATACAATATT CCTTTAGTAT CAGGTCCTGA TATACCCATT AATATAACTG ACCAAGCTCC
TTGGTGGTGC ATACAATATT CCTTTAGTAT CAGGTCCTGA TATACCCATT AATATAACTG ACCAAGCTCC
TTGGTGGTGC ATACAATATT CCTTTAGTAT CAGGTCCTGA TATACCCATT AATATAACTG ACCAAGCTCC
TTGGTGGTGC ATACAATATT CCTTTAGTAT CAGGTCCTGA TATACCCATT AATATAACTG ACCAAGCTCC





```

P#3 Clone
Sequencher™ "Min. vector"

 P3-m13F
 P3-m13R
 P3-m13R
 HpV16-L2.Seq
 #421





TTCATTAAATT CCTATAGTTC CAGGGTCTCC ACAATATACA ATTATTGCTG ATGCAGGTGA CTTTATTTTA
 TTCATTAAATT CCTATAGTTC CAGGGTCTCC ACAATATACA ATTATTGCTG ATGCAGGTGA CTTTATTTTA
 TTCATTAAATC CCTATAGTTC CAGG
 TTCATTAAATT CCTATAGTTC CAGGGTCTCC ACAATATACA ATTATTGCTG ATGCAGGTGA CTTTATTTTA

TTCATTAAATT CCTATAGTTC CAGGGTCTCC ACAATATACA ATTATTGCTG ATGCAGGTGA CTTTATTTTA

 P3-m13F
 P3-m13F
 P3-m13R
 HpV16-L2.Seq
 #491

ACATGTT ACGAAAAACGA CGTAAACGTT TACCATAATTT TTTTTCAGAT GTCTCTTTGG
 CATCCTAGTT ATTACATGTT ACGAAAAACGA CGTAAACGTT TACCATAATTT TTTTTCAGAT GTCTCTTTGG
 CATCCTAGTT ATTACATGTT ACGAAAAACGA CGTAAACGTT TACCATAATTT TTTTTCAGAT GTCTCTTTGG
 CATCCTAGTT ATTACATGTT ACGAAAAACGA CGTAAACGTT TACCATAATTT TTTTTCAGAT GTCTCTTTGG

CATCCTAGTT ATTACATGTT ACGAAAAACGA CGTAAACGTT TACCATAATTT TTTTTCAGAT GTCTCTTTGG

 P3-m13F
 P3-m13F
 P3-m13R
 HpV16-L2.Seq
 #561

CTGCCTAGAG ATCTGGAGGG CGGATCCCC GGGCTGCAGG AATTCGATAT CAA
 CTGCCTAGAG ATCTGGAGGG CGGATCCCC GGGCTGCAGG AATTCGATAT CAA
 CTGCCTAGAG ATCTGGAGGG CGGATCCCC GGGCTGCAGG AATTC
 CTGCCTAGAG ATCT

CTGCCTAGAG ATCTGGAGGG CGGATCCCC GGGCTGCAGG AATTCGATAT CAA

Investigator

Jessica Ling

Date

25 Nov 97

Subject

Ligation #12/Transformation

Filed in Book Number/Title

Ling HPV-1 / JCL 97-103

Setup ligation #12

Another attempt for a 3way ligation into
one yeast vector

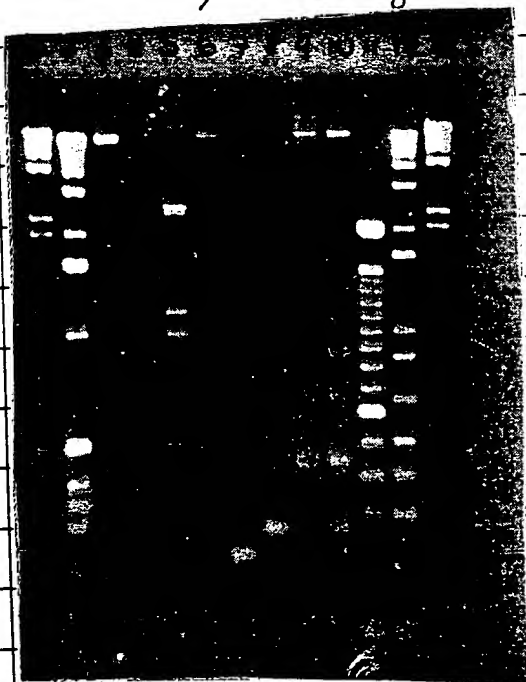
I/C' (Sma I/Not I) JCL 97-59

A'/D2 (Not I/Bgl II) JCL 97-96

TUBE #	Vector Name	# μ L	Insert Name	# μ L	10 x Buffer	T4 DNA Ligase	# μ L dH ₂ O
1	D03 (Sma I/Bgl II)	1ul	\emptyset	\emptyset	2ul	1ul	16ul
2	I	I	VIN: AgB3 (PCI)	2	I	I	14ul
3	I	I	I/C' A'/D2	4/4	I	I	8ul
4	I	I	I/C' A'/D2	6/6	I	I	4ul

Incubate o/n @ 16°C

26 Nov 97

PCI of ligation, EtOH ppt (BMB # 83628520)
Resuspend into 20 μ L dH₂O
load 10 μ L onto gel

1. Lambda Hind3 - 5ul

2. 1 kb ladder - 5ul

3. D-037 linker - 1ul

4. Lig ①

5. VIN: AgB-3 - 1ul

6. Lig ②

7. I/C' (Sma I/Not I) - 2ul

8. A'/D2 (Not I/Bgl II) - 2ul

9. ③ lig

10. Lig ④

11. 100 bp ladder - 5ul

12. 1 kb ladder - 5ul

13. Lambda Hind3 ladder - 5ul

Jessica Ling
26 Nov 97

Countersigned by

Cornelia De Jijl

Date

22 Jan 98

DO NOT BEGIN NEW EXPERIMENTS ON THIS PAGE.

Transform into DH5 (# JTB703 Gibco) 25ul

① 24

② ~1-200 colonies

③ 30ul = 13 70ul = 39

④ 1 = 14 = 32

30ul of total 100ul transform

70ul of 100ul transform

Plated onto Remel LBAPP #5639 exp 12-16

Pick + patch colonies.

Jessica
26 Nov 97

Investigator

Jessica Ling

Date 02 Dec 97

Subject

PCR screen of JCL 97-103

Filed in Book Number/Title

Ling HPV-1 / JCL 97-110

Setup clonal screen (96) of JCL 97-103
(3 way lig)

COMPONENT	Manufact/Lot Number	[FINAL]	1 X VOLUME	? X VOLUME
				110x
DNA -- Clones			20µL	—
10 x Buffer	Perkin Elmer/ #G0874	1 X	5µL	550ul
25mM MgCl2	Perkin Elmer/ #G0873	3.0mM	6µL	1660ul
10mM dNTPs	Stratagene/ #0276151	0.8µM	4µL	440ul
5µM Primer #1	233 I	0.2µM	2µL	220ul
5µM Primer #2	233 D2	0.2µM	2µL	220ul
Taq Gold	Perkin Elmer/ #G1092	1.25U	0.2µL	27.5ul
dH2O	RCM66/ #M1-7113		10.73µL	1182.5µL
Total			50µL	Aliquot 30µL/Tube

JCL 02 Dec 97

Res. Method:

Results:

① 95°C - 10'

Positives # (10/96)

② 94°C - 45"

⑪

75

60°C - 45"

30X

26

⑦⑨

72°C - 45"

②⑨

⑧①

③ 72°C - 10'

④④

84

④ 4°C hold

71

95

Circled clones will be grown up in
100ml for DNA prep

Jessica Ling
02 Dec 97

Countersigned by

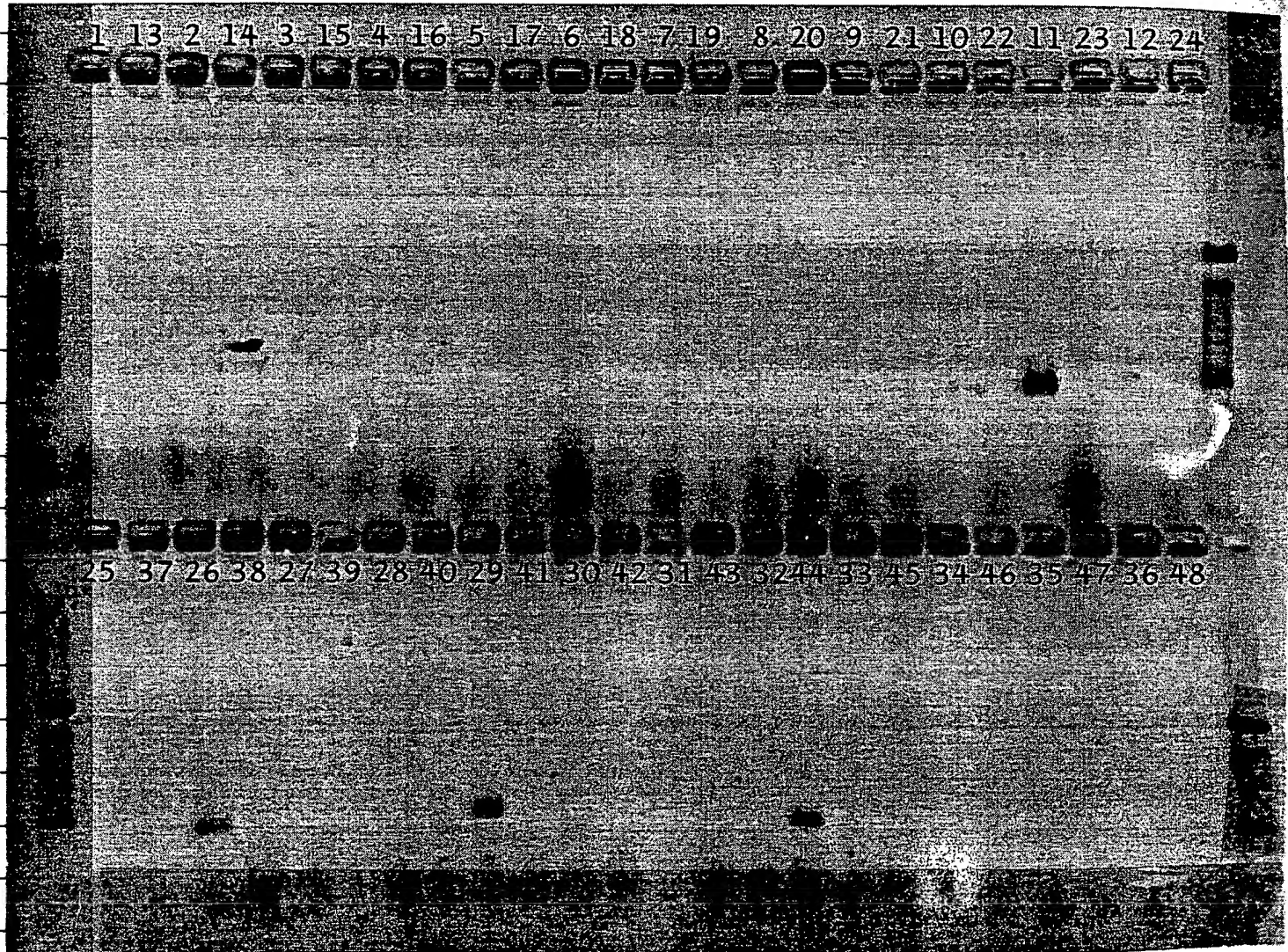
Corinne De Mui

Date

22-Jan-98

DO NOT BEGIN NEW EXPERIMENTS ON THIS PAGE.

JCL 97.110
3-Way Ligation Colony Screen
(pGAL110) 3WID#1-48



Jessica R
02 Dec 97

Book 16344

Page

251

Project No.

Project Page

Investigator

Jessica King

Date

02 Dec 97

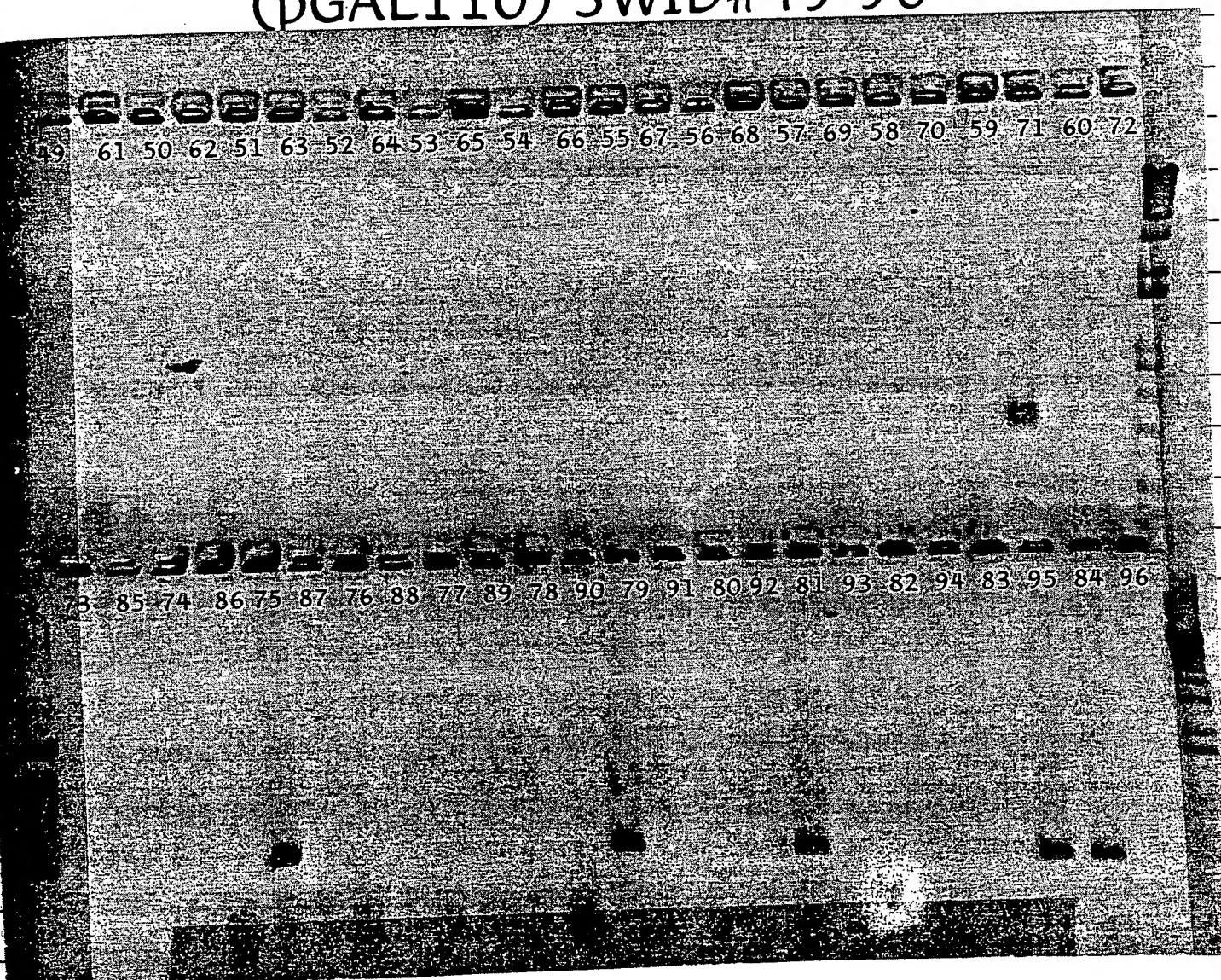
Subject

Colony screen JCL 97-103

Filed in Book Number/Title

King HPV-1 / JCL 97-110 Continued

JCL 97-110
3-Way Ligation Colony Screen
(pGAL110) 3WID#49-96



Jessica King
02 Dec 97

Corrine DeNitt

Date

20 Jan 98

Investigator

Date

Oct 9, 1997

Subject

Mutagenesis PCR amplification of 16E2 from HPV

Filed in Book Number/Title HPV4

Purpose: Express 16E2 in yeast linked to L2 want to see if incorporated into virions. Must first KNOCK OUT 2 amino acids responsible for E2 function

Materials:

"Wildtype 16E2" Clone 1415 from J. Zhang
Oligos see E257A for what was ordered & changes made to Gene

Methods:

Reference paper for Mutants = J. Virology Vol 70 (3) 1602-1611
Sakai, Hiroyuki; et al

PCR out inserts as 3 PCR products

	LA Tag			Ampl. Tag (BmB)		
	(1)	(2)	(3)	(4)	(5)	(6)
H ₂ O	83.5	83.5	83.5	83.5	83.5	83.5
10x buffer + Mg	10	10	10	10	10	10
25mM dNTP	2	2	2	2	2	2
Primer: Top 100%	1.5 MN492	1.5 MN494	1.5 MN496	1.5 MN492	1.5 MN494	1.5 MN496
Bottom 100%	1.5 MN493	1.5 MN495	1.5 MN497	1.5 MN493	1.5 MN495	1.5 MN497
Template	1	1	1	1	1	1
Tag Pol. 50/1	0.5	0.5	0.5	0.5	0.5	0.5
	100	100	100	100	100	100

94°C, 1 min

94°C, 30"

50°C, 30"

68°C, 30"

Linked +4°C Overnight

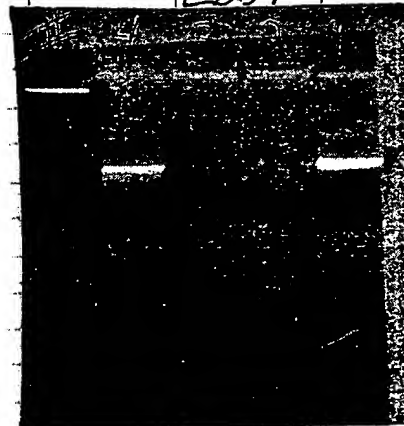
18 MN Oct 9, 1997
20 cycles

Note All rxns performed in duplicate

XhoI Not

Note: Must blunt end this in not to destroy sites

E257-



Results:

See gel - Should be

#1 = 160 bp

#2 = 135 bp

#3 = 900 bp

#4 = (same as #1 but using Tag Pol.)

COPY OF REPORT

Countersigned by _____ Date _____

Investigator Michael JoyceDate 12/11/97Subject Insertion of Dead 16EZ Mutant into 16LZ (minimal) expression.Filed in Book Number/Title HPV4

Purpose: Insert 16EZ of dead mutant (E280) into LZ for expression and to see if incorporated into VLPs.

Materials:

Received YPB-1 (yeast pGAL110 HPV16 L1, LZ minimal vector) from J. Ling.

E280-6 NotI XhoI frag from This notebook
E280-8 " " " "

Methods:

NotI, XhoI Digest

✓ DNA
✓ 10x H₂O buffer
✓ H₂O
XhoI
37°C, 30 min
NotI

37°C ~ 2 1/2 hr

YPB-1 vector
①
100
10
10
70
5
95
5
100

E280-6
②
20
10
80
5
95
5
100

E280-8
③
20
10
80
5
95
5
100

Inserts
-1 (2ul)
-2 (2ul)

E295-1
NotI XhoI
vector (2ul)

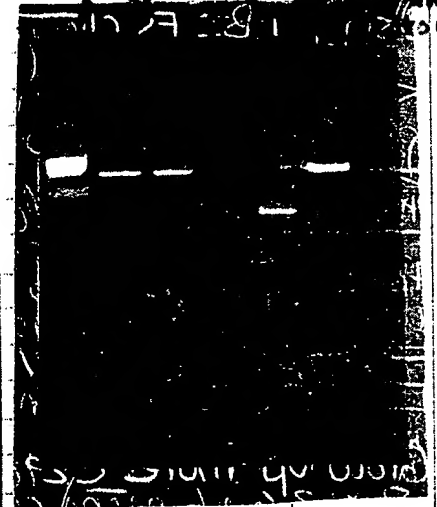
Gel on 1% LMP Agarose (Inserts only)

Vector: Ethanol ppt.

Shrimp alkaline phosphatase treatment
E295-1 Not XhoI digest

44 µl H₂O (DNA)
5 µl 10x buffer
0.5 µl Shrimp Alk Phos.
50

37°C 1 hr.



Phenol extract -1 -2 + -3 1x CHCl₃ extract 1x and Eth. ppt
Resuspend in 20 µl H₂O Run on gel above

Ligate as Reverse (E296)

DO NOT BEGIN NEW EXPERIMENTS ON THIS PAGE.

Ligation:

	①	②	③
✓ vector YIF3-1 Not XhoI shrimp NK ₂ 5	5	5	5
✓ insert	4 (E295-2)	4 E295-3	0
✓ 10x Dig. buffer BMB	1.5	1.5	1.5
✓ H ₂ O	3.5	3.5	7.5
✓ 14 DNA ligase	1	1	1
	<u>15</u>	<u>15</u>	<u>15</u>

15°C O/N

Transform DHS cells as usual (BRL comp. cells)
 plate LB Amp Pker Wknd @ 28 C

M. J. J. 12/19/97

Pick colonies from ligation rxns E296-1 & E296-2 lig since the colony count was so high relative to the no insert control ligation plates (approx. 5-10x more cfus on -1 & -2 vs. -3)

Pick 4 clones from each plates E296-1 & 2 (lost 2 clones during isolation isolate DNA from O/N cultures in LB Amp using Promega Midiprep SV (3.6 ml culture). Followed their procedure, did not ethanol ppt before sequencing.

Digest clones w/ Not XhoI to confirm presence of ~1.2 Kb 16EZ in

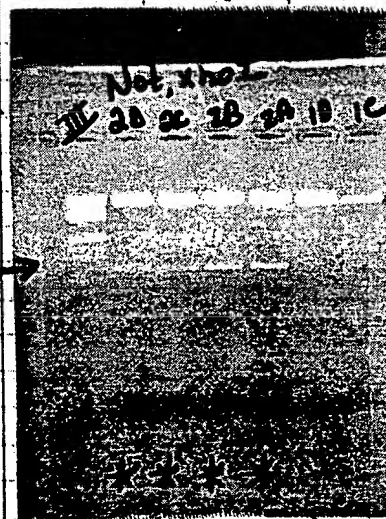
Picked clones 2A & 2B for sequencing

Using ABI FS Chem. Terminator dye mix as standard using 6 µl (of 100 µl) DNA from each prep. See sections #14 & 15 of book #4 Sequencing & gel files on ZIP100 drive

Results:

E296-2B Sequence appears to be good with no changes that would affect AA translation.

M. J. J. 12-19-97



Grow up more E296-2B isolate using 8 x 3.6 ml preps of Promega Wizard SV Miniprep 70% k.t. Then phenol/CHCl₃ extract, Ethanol ppt wash & dry Resuspend sterile water for yeast transformation

Give to B. McClements

correct clones

M. J. J. 12-19-97

Countersigned by

B. A. Arnold

Date

2/3/98

Investigator Michael J. ArnoldDate 12/15/97Subject Insertion of 16E7 into pMB-1 Minimal L2 VectorFiled in Book Number/Title HPV4

Purpose: Insert 16E7 into middle of 16L2 coding region of Minimal L2 org.

Materials:

16E7 DNA from 1415 clone from J. Zhang

Methods:

PCR of using primers MNS12/MNS13

	①	②	③	④	
H ₂ O	40.5				
10x PCR buffer w Mg	5				
10 mM dNTPs (BMB)	2				
Primer MNS12 500 ng	0.5				
MNS13 500 ng	0.5				
Tag Pol	0.5				
Template 1415 16E7	1.0				
94°C 1 min	50				
94°C 30"					
55°C 30"					
72°C 30"					
15 cycles					
7 min 72°C					
4°C hold.					

Rxn in quadrup

From the 4 rxns above pooled & run
Gel isolate 0.3 Kb frags on 1.8% LMP (SeaPlaque) agarose
cut appropriate band and digest w/ Apsase (BMB) 45°C for 1.5 hr
Phenol/CHCl₃ ppt resusp. in 200 µl H₂O
MNS12/MNS13 160 µl H₂O use half in each of 2 tubes as below

Not XhoI Digest (Done in 2 tubes)

DNA	80	37°C, 2 hr (tube 1)
10x H ₂ O buffer	10	37°C, Overnight (tube 2)
H ₂ O	0	
Not I	5	
Xho I	5	
	100	

Phenol/CHCl₃ ppt wash dry resuspend tube 1 in 20 µl H₂O
Use for ligation E298.

DO NOT BEGIN NEW EXPERIMENTS ON THIS PAGE.

Ligation:

Vector: E295-1 NotI xhoI "Shrimped"
 Insert: E297-tube 1 NotI xhoI 16E7

BMB
Reagents

10X lig buffer (w/ ATP)

H₂O
T4 DNA ligase

14°C O/N

Transform DHS cells as usu. (See E296)

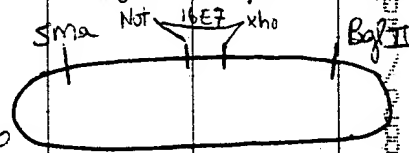
Pick 9 transformants, grow cultures overnight 37°C LB Amp

Isolate DNA - Promega Wizard SV 3.6 ml culture Harvest 100 µl H₂OAnalysis: Bgl II, Sma I digest.

Above digest would release a ~550 bp
 frag if no 16E7 was present or ~900 bp
 if 16E7 was located w/in Bgl II Sma I

Digest: DNA 7
 10x A bu 2
 H₂O 9
 Bgl II 1
 Sma 1
 20

PCR Conditions for Digest:
 37°C, 30'; 25°C, 30'
 Run on 1.2% Agarose

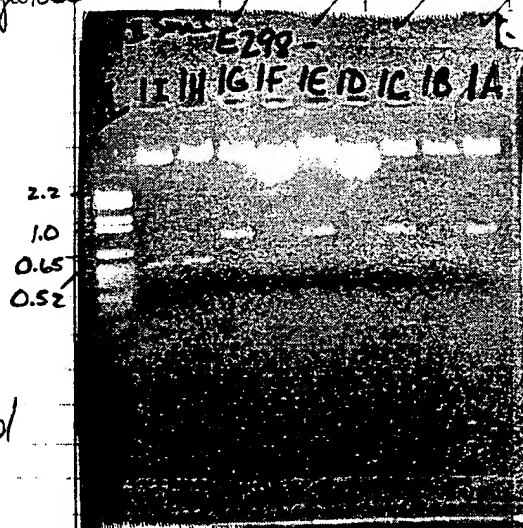


Correct clones = E298-1A, 1C, 1E, 1G
 Sequence w/ FS chem (ABI) w/ DNA
 (See section 16 of Seq Book #4)

Results:

Sequence of clone E298-1G
 appears correct (2 other clones had
 changes and 1 other clone lacked good
 sequence reads in both directions)
 (Primers from J. Ling - See Seq book #4)

Isolate DNA from E298-1G using Promega Wizard SV prep
 then phenol CHCl₃ Ethol ppt wash dry.
 Resuspend in 30 µl sterile dH₂O Rant60; Provide 25 µl to McClements, W.
 for transformation of yeast



Project for

Jan S, 1998

Investigator Michael H. [Signature]

Date

Dec 29, 97

Subject Preparation of 16E7 and Insertion into Host L1 + L2 vector.

Filed in Book Number/Title

HPV 4

Purpose: Inserted 16E7 orf into NotI XhoI of L1 + L2 minimal vector (= construct E299).
Now want to insert 16E7 into the full length L2 orf at NotI and XhoI sites.

Materials:

vectors (from J. Ling)

YGH#3

(JCL97.131)

Nuclear Localization Signal (L)

YFG#5

(JCL97.131)

full length L2

LA vector

Invitrogen (for subcloning)

insert:

16E7 template = 1415 from J. Zhang

primers = MNS12 MNS13 from J. Midland Reagent Co.

Method:H₂O

10x Tag buff w/ Mg (BMB)

10 mM dNTPs (BMB)

Primer: MNS12

MNS13

Template

Tag Pol su/x (BMB)

1x

40.5

5

2

0.5

0.5

1.0

0.5

50

(did 4 of these reactions for more final product)

Cycles:

94°C

55°C

72°C

30" each

15 cycles

7 min 72°C hold → 4°C ∞

Store -20°C

12/29/97

12/31/97

Gel separate 1.8% LMP Agarose then gel w/ by Agarose (BMB) Method
Resuspend 30 µl. = E299-10Digestion:

Cut 1/3rd w/ XhoI, 1/3rd w/ NotI, 1/3rd Uncut

DNA

10x H buffer

H₂O

XhoI

10

7

46

7

70

37°C, 2 hr

DNA

10x H buffer

H₂O

NotI

10

7

46

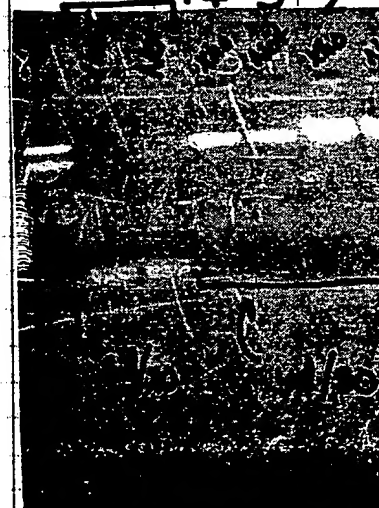
7

70

12/31/97

E299-1 E300-

4 3 7



Beth A. Arnold

2/3/98

Vector Prep:

YFG#5

VGH#3

15	15
10	10
65	65
10	10
<u>100</u>	<u>100</u>

Not I Xho I

15	15
10	10
65	65
10 NaI	10 X ₂ O ₂
<u>100</u>	<u>100</u>

Note: Since DNA appears viscous⁺ - I am doubling above volumes for each to 200ul by addition of: 10ul 10x buffer, 5ul more enzyme, 85ul H₂O
 Bind: CHCl₃, ppt resuspend 44ul H₂O

Shrimp ALK Phosphatase see E295 for Materials & Method of use

DNA 44/4
10x dig 5
Salt mp NR 0.5
50

Note: Doubled recipe here too to 100ml as this is large plasma and at high concn/pt.

37°C	1 hr
------	------

Phend' CHCl_3 ppt Resusp. 30ul (See gel E299)

Full length

YFG#S' vector

$\psi_{GH\#3}$ vector

LA vector

Ligation:

- ✓ Vector
- ✓ insert
- ✓ 10x Dlgbuf
- ✓ H₂O
- T₄ DNA ligase

A	B	C	D
(A)	(B)	(C)	(D)
3	3	6	6
4	4	4	4
2	2	2	2
10	10	7	7
1	1	1	1
20	20	20	20

2 TA Vector (Invitrogen)
 2 E29-1
 undig
 1
 ✓ 4
 1
 10
 E29-1
 x
 E29-1
 N
 E300
 4 3 2 1

14°C, until 1/5/98

5-Jan 98

Transform Gibco DHS cells per Manufact.
Plate Remel LB Amp plates @ 37°C O/N

5- Tang 8

Countersigned by

Date _____

2/3/98

Book 16344

Page 27

Project No.

Project Page

Investigator

Date

08 Dec 97

Subject

Sequencing of YP3-1, 2, 70 and 72

Filed in Book Number/Title

Long HPV-2 / JCL 97-118

Setup Sequencing Run for YP3 #1, #2, #70 & #72

Use: L2/184F as primers.

L2/167R

L2/2466F

L2/157F

L2/45R

L2/1261R

L2/1384F

L2/1339F

L2/16411

dilute DNA to [0.25 µg/µl]

DNA - 3 µl

EStag - 4 µl

primers 5 µl / 1 µl each

1.1 µl / 3.2 µl

dH₂O

11.9 µl / 9.8 µl

Results:

Looked at YP3 #1 and YP3 #2 under analysis program. Both looked very good.

Will hand YP3 #1 to Bill McLemerts as minimal vector. Also will give to Mike Neepu to clone E proteins.

Jessica
10 Dec 97

Countersigned by

Concetta Desjard

Date

06 Feb 98